MODIFIED LABORATORY TECHNIC FOR PREPARING CHROMOSOMES FROM HUMAN AND ANIMAL PERIPHERAL BLOOD LEUKOCYTES

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FOREWORD

This report was prepared by the following personnel in the Pathology Department:

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ABSTRACT

A method for obtaining chromosome spreads from human and animal blood is presented in detail. It is based on previously described methods which have been modified to improve the chances of successful culture and to provide a more flexible system for clinical and experimental purposes.

This technical documentary report has been reviewed and is approved.

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1. INTRODUCTION

Technics for obtaining chromosome preparations from peripheral blood are a relatively recent development (1, 2). Such preparations have been used extensively in the study of various clinical disease states and familial abnormalities in man (3-7). Similar technics have also been applied in the study of chromosomes in the blood cells of animals (8).

Despite the fact that the chromosome technic is being widely applied in clinical medicine, it has not been rigidly standardized from laboratory to laboratory and is still in the developmental stage. The method described here is a modification of previously reported technics for both experimental and clinical usage. The use of multiple tubes, in particular, has proved to be of great advantage in assuring the successful culture of cells.

A detailed presentation of the technic should be of considerable help to laboratory personnel who have had no previous experience with the preparation of leukocytes for chromosome studies.

2. MATERIALS

The following materials are required:

1. Heparin sodium, 1,000 U.S.P. units/ml.

2. Phytohemagglutinin M. Sterile distilled water, 5 ml., is added to each vial.

3. Medium 199.

4. Penicillin-streptomycin mixture, 5,000 units/ml.

5. Colchicine. Make up to 1 : 10,000 in Tyrode's solution.

6. Orcein, synthetic (2 gm. of orcein diluted with 50 ml. of 45% acetic acid containing 10 ml. of absolute alcohol). This is boiled under a reflux condenser for 5 minutes and allowed to cool. It is then filtered and another 50 ml. of 45% acetic acid added.

3. METHODS

Preparation of cell cultures

All glassware and solutions used in the cultures must be thoroughly acid-washed and sterilized. If careful sterile technic is observed, it is not necessary to be overly elaborate, and the use of sterile gown, mask, and gloves and a "sterile room" only makes the procedures more difficult and time-consuming. The most important source of contamination in this laboratory has been the phytohemagglutinin, which occasionally contains yeast. Contamination of this material has been found only when the phytohemagglutinin solution has been kept for several weeks.

Venous blood, 2 ml. or more,[1] is drawn into a sterile syringe wetted just previously with the heparin solution. It is then placed in a conical centrifuge tube to which additional heparin in a concentration of 0.1 ml./10 ml. of whole blood has been added.

The centrifuge tube is placed at an angle of 60° and allowed to stand at room temperature (approximately 27° C.) for 90 minutes. Phytohemagglutinin M (0.1 ml./10 ml. blood) is then added and the sample placed in the refrigerator (approximately 4° C.) at a 60° angle for 30 minutes.

[1]When it has not been possible to obtain a large volume of blood, cultures have been successfully prepared from as little as 2 ml. of whole blood. The chances of success are definitely greater with a larger volume, however. Ten ml. of blood is suggested as being the desirable quantity to use in most instances.

After being refrigerated, the specimen is centrifuged at 21 R.C.F.[2] for 5 minutes, and the supernatant plasma containing the leukocytes is removed with a sterile syringe and a thoracotomy needle which has been cut through at right angles to the shaft to give a blunt tip. Care should be taken not to disturb the red cell layer, although a few erythrocytes in the final mixture do not appear to have an adverse effect. Mild hemolysis does not interfere with the cultures, but marked hemolysis is inhibitory and must be avoided.

The aspirated plasma and cells are diluted with Medium 199 in a proportion of 1 part plasma to 4 parts medium. Medium 199 is prepared to contain penicillin, 50 units/ml., and streptomycin, 50 μg./ml.

The final culture suspension is mixed thoroughly but gently. Two ml. aliquots are placed in 15 ml. screw-cap test tubes 125 mm. in length and 15 mm. in diameter; the cap is tightened, and the tubes are incubated at 37° C. If possible, several tubes should be prepared, to allow selection of the most promising cultures for preparation of smears.

Preparation of chromosome spreads

Most cultures will be ready for processing at the end of 72 hours of incubation, although longer incubation is occasionally required. Two or three tubes are harvested at 72 hours. The remaining tubes are kept at 37° C. until 96 hours, and then processed if the first group proves to be unsatisfactory.

Two drops of 10^{-6} M colchicine are added to each tube to be harvested, and incubation at 37° is continued for 2 to 4 hours. The cells are suspended by trituration, transferred to centrifuge tubes, and centrifuged for 5 minutes at 40 R.C.F. Most of the supernatant is discarded, leaving only enough liquid to suspend the cells thoroughly. One ml. of 1% sodium citrate, warmed to 37° C., is added to each tube; at the end of 5 minutes 1 drop of fresh fixative (3 : 1 absolute ethanol–glacial acetic acid mixture) is added and the mixture shaken. This is centrifuged for 5 minutes at 40 R.C.F., most of the supernatant is decanted, and the remainder shaken gently. Two ml. of the fixative is added slowly with mild shaking, any clumps present are broken with gentle pipetting, and the suspension is allowed to stand for 5 minutes. The suspension is then centrifuged for 5 minutes at 230 R.C.F., and the fixative replaced by 2 ml. of fresh fixative. This is allowed to stand for 15 minutes and recentrifuged at 230 R.C.F. It may be desirable to repeat this step a few times to improve the quality of the spreads. The final suspension should be cloudy in appearance.

A small drop of the suspension is placed in the center of a thoroughly cleaned microscope slide and spread by gently blowing upon it. The drop will spread to a diameter of 1 to 1.5 cm., and should be blown until dry.

The spreads are stained with a 2% solution of orcein for 15 minutes and rinsed twice in separate containers of absolute ethanol. They are then placed in a 1 : 1 absolute ethanol-xylol mixture for 1 minute, immersed several times in three separate containers of xylol, and mounted with Permount. This staining procedure has proved quite satisfactory, although other stains such as the Fuelgen stain may be used.

Microscopic examination and photography

Microscopic examination may be done on specimens stained as described above, using either a bright field or phase contrast system. In the most desirable preparation, the individual chromosomes are straight and not overlapping each other, and the cells are distinctly separated. Photomicrographs of chromosome spreads obtained from human and rabbit blood are illustrated in figures 1 and 2. A Leitz Ortholux microscope with a Leitz Aristophot photographic attachment is used in this laboratory, although smaller-size sheet film or even 35 mm. roll film can be used if the film type is properly selected and the focus is exact. A high contrast black-and-white film, such as Kodalith Pan, has proved most satisfactory.

[2]The relative centrifugal force of a particular centrifuge is calculated from the formula: $R.C.F. = (1.118 \times 10^{-5}) \times r \times N^2$, where r = the rotating radius and N = number of revolutions per minute.

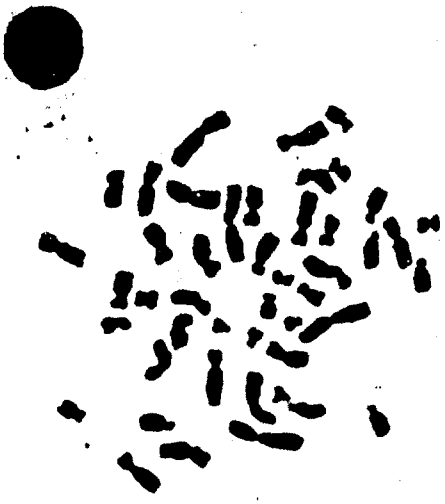

FIGURE 1

Chromosome spread from human peripheral blood.

FIGURE 2

Chromosome spread from rabbit peripheral blood.

4. DISCUSSION

Interpretation of the results is made primarily on the basis of total chromosome counts in at least 50 cells (if possible) and on the basis of the morphologic appearance of the individual chromosomes. Karyograms of several cells are made, with the grouping arranged according to the Denver system (9, 10). Figure 3 illustrates a karyogram of the spread shown in figure 1. We have employed a similar method for grouping animal chromosomes, as shown by figure 4, which represents a karyogram of the spread in figure 2. Such grouping

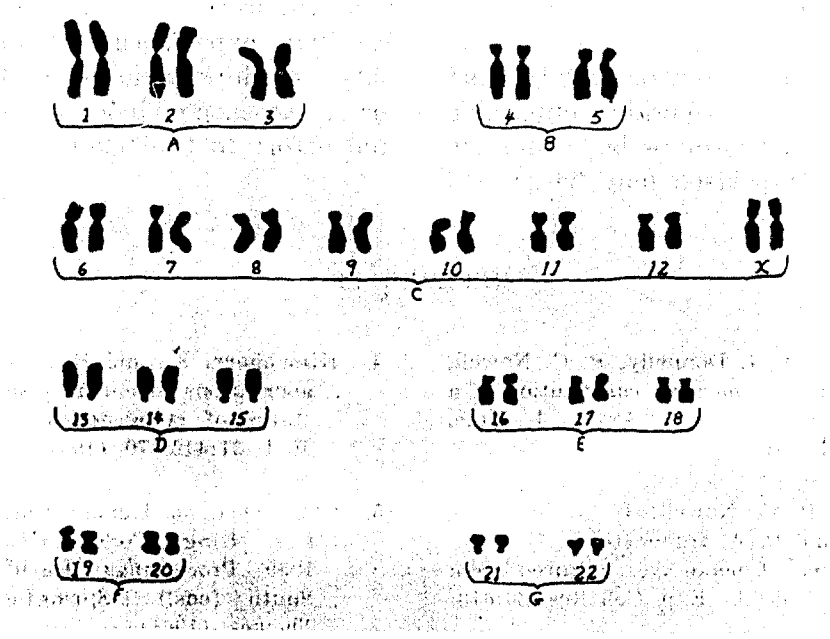

FIGURE 3

Grouping of the chromosomes from figure 1 following the Denver system.

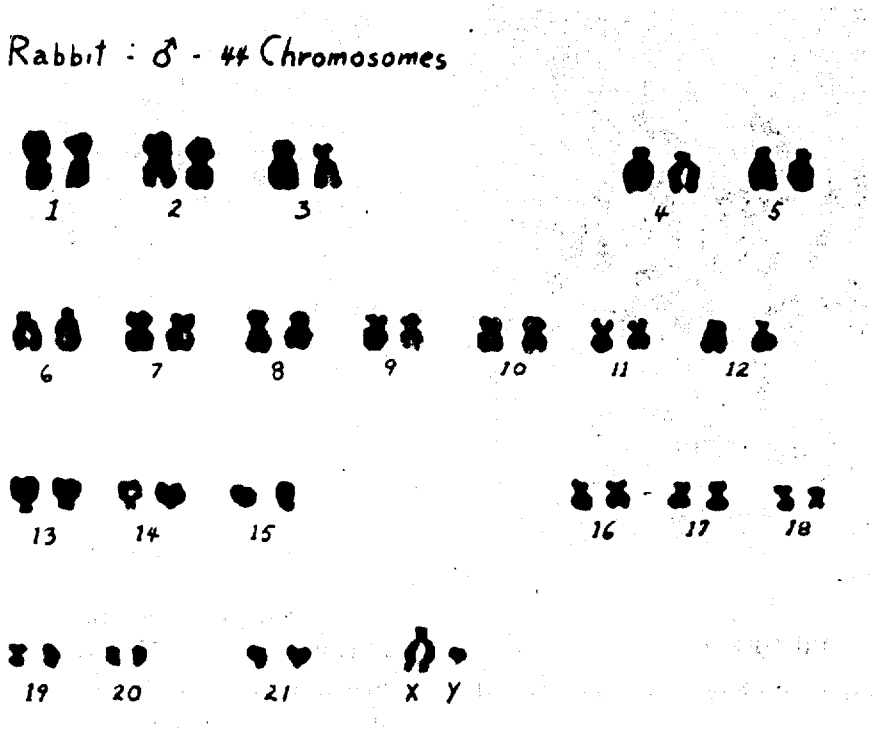

FIGURE 4

Grouping of the chromosomes from metaphase cell in figure 2.

allows a more accurate classification of numbers and morphology than can be made with the microscope alone.

The total technical and professional effort required for this technic is roughly comparable to that required for a thorough bone marrow examination. The comparison may be carried further, to say that it is highly desirable to have the blood drawn by the same personnel who will process and interpret the preparations. We have experienced numerous failures when this procedure could not be followed, at considerable expense of blood to the patient and time and effort to the technicians.

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2. Chromosome analysis

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